

A CRAB-HOLE MOSQUITO, *OCHLEROTATUS BAISASI*, FEEDING ON MUDSKIPPER (GOBIIDAE: OXUDERCINAE) IN THE RYUKYU ISLANDS, JAPAN

HARUO OKUDO,¹ TAKAKO TOMA,² HITOSHI SASAKI,³ YUKIKO HIGA,² MAYUMI FUJIKAWA,³ ICHIRO MIYAGI² AND TAKAO OKAZAWA⁴

ABSTRACT. *Ochlerotatus baisasi* was observed and photographed feeding on a mudskipper (*Periophthalmus argentilineatus*). Origin of blood meals in the stomachs of female mosquitoes collected at burrows of land crabs and mud lobsters in the mangrove forests of Oura, Okinawa, and Komi, Iriomote (the Ryukyu Islands), was determined by enzyme-linked immunosorbent assay to be from a fish source.

KEY WORDS *Ochlerotatus baisasi*, feeding behavior, blood meal source, mudskipper, *Periophthalmus argentilineatus*, Ryukyu Islands

INTRODUCTION

In the Ryukyu Islands of Japan, adult and immature of *Ochlerotatus* (*Geoskusea*) *baisasi* (Knight and Hull) are commonly found in holes made by land crabs of the family Grapsidae and the mud lobster, *Thalassina anomala*, near mangrove forests (Miyagi and Toma 1978, Tanaka et al. 1979, Miyagi and Toma 1980, Mogi et al. 1984, Toma and Miyagi 1986, Ng and Kang 1988). However, no information is available on the blood meal source and the feeding behavior of this mosquito.

According to Belkin (1962), 9 species of mosquitoes belonging to the subgenus *Geoskusea* have been reported from South Pacific Islands and Southeast Asia—*tonus* (Edwards) and *kabaensis* (Brug) from Indonesia; *baisasi* from the Philippines and Ryukyu Islands, Japan; *fimbripes* (Edwards) from New Guinea and the Bismarck Archipelago; *daliensis* (Taylor) from northern Australia; *becki* (Belkin), *perryi* (Belkin) and *longiforceps* (Edwards) from the Solomon Islands; and *daggyi* (Stone and Bohart) from Vanuatu. All species use holes made by land crabs near the coast for breeding and resting. *Ochlerotatus* (*Geoskusea*) *longiforceps*, which is closely related biologically and morphologically to *Oc. baisasi*, has been reported to bite mudskipper (*Periophthalmidae*) on Gizo Island, in the Solomon Islands (Stooff and Marks 1965). To our knowledge, although several mosquito species have been reported to feed on reptiles and amphibians (Reington 1945, Henderson and Senior 1961, Hayes 1965, Nalan et al. 1965, DeFoliart 1967, Miyagi 1972), feeding on fish is quite rare.

Ochlerotatus longiforceps is the 1st authentic record of a mosquito biting a fish (mudskipper).

The use of immunological techniques to identify blood meals is a valuable aid in determining the blood source animal (Sasaki 1988). This paper presents observations on the feeding behavior of *Oc. baisasi* in mangrove forests in Oura, Okinawa, and Komi, Iriomote (Ryukyu Islands), and in the laboratory as well as the results of enzyme-linked immunosorbent assays (ELISAs) of blood meals in the stomachs of wild-caught mosquitoes.

MATERIALS AND METHODS

Observation on biting behavior of *Oc. baisasi* vis-à-vis mudskipper in the laboratory: Mosquito larvae were collected from crab holes in the mangrove forest of Oura on September 21, 2002, by hand pump and reared to the adult stage in the laboratory. A mudskipper (*Periophthalmus argentilineatus* Valenciennes) also was collected at a tide pool in the mangrove forest of Oura. The mudskipper was confined in a cage (20 × 20 × 30 cm) with about 148 newly emerged female and male mosquitoes. The cage was placed outdoors, and the feeding activity of the mosquitoes was observed from October 3 to 16, 2002, through a close-up lens attached to a camera. Engorged mosquitoes were transferred to another cage of the same size. A tube (15 × 5 cm diameter) made of black cardboard served as a simulated crab hole for resting and oviposition.

Blood meal determinations in the stomachs of wild-caught female mosquitoes by ELISA: Resting female *Oc. baisasi* engorged with fresh blood and half gravid were collected by sucking tube from the upper parts of crab holes in the mangrove forests of Oura and Komi. Twenty-seven engorged specimens were squashed individually onto a filter paper (Whatman No. 1; Whatman, Tokyo, Japan) immediately after capture. The filter papers were kept in a desiccator with silica gel after being air dried in the field, and the smeared parts were cut off with scissors and stored individually in a microvial until assaying. The ELISA procedure for determination

¹ Miwa Junior High School, 519 Makabe, Itoman, Okinawa 901-0336, Japan.

² Laboratory of Medical Zoology, School of Health Sciences, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan.

³ Laboratory of Entomology, Rakuno Gakuen University, Bunkyoudai-midirimachi, Ebetsu, Hokkaido 069-8501, Japan.

⁴ International Student Center, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan.

Table 1. Feeding habits of *Oc. baisasi* on a caged mudskipper, *P. argenteolineatus*.^{1,2}

Case no.	Feeding location	Time feeding took place (h)	Remarks
1	Between eyes	~ 2200	Penetrated tissue but no bloodfeeding took place
2	On the head	~ 2200	Bloodfeeding interrupted by host movement, mosquito returned and completed feeding
3	Between eyes	~ 2200	Flew away from the host immediately after full bloodfeeding
4	On the pectoral fin	~ 2200	Flew away from the host immediately after full bloodfeeding
5	On the head	1947	Feeding interrupted by host, no blood taken
6	Near mouth opening	2007	Feeding interrupted by host, no blood taken
7	On the head	2031	Rested on the host for a few minutes after full bloodfeeding accomplished
8	On the back near the dorsal fin	1952	Rested on the host for a few minutes after full bloodfeeding accomplished

¹ Average time per blood meal was 10 min.

² Experiments were conducted October 3–16, 2002.

of blood meal source was that of Sasaki (1988), with the use of anti-fish (serum) sheep serum (Cosmo Bio Co., Ltd., Tokyo, Japan) and HRP-conjugated anti-sheep (IgG) rabbit serum (ICN Pharmaceuticals, Inc., Costa Mesa, CA) as a conjugate.

RESULTS AND DISCUSSION

Eight observations of feeding activity are shown in Table 1. Male and female *Oc. baisasi* seem to be nocturnal or crepuscular in activity, resting by

day in the black paper tube provided in the cage. Mating swarms were observed to take place above the opening of the tube at dusk and dawn. Some female mosquitoes were observed to hover over the mudskipper and alight on the dorsal part of the fish, walking around and eventually feeding between the dorsally protruding eyes or the pectoral fin musculature of the mudskipper.

Feeding usually lasted about 10 min for a full bloodfeeding. The female appeared to be motionless during the feeding, and the fish did not appear



Fig. 1. *Ochlerotatus baisasi* feeding on a mudskipper, *P. argenteolineatus*.

Table 2. Number of *Oc. baisasi* for which the blood meal was determined by ELISA to be from fish.

Collection site	Collection date (2002)	Engorged females testing positive for a fish blood meal	Engorged females testing negative for a fish blood meal
Oura, Okinawa	July 6	1	0
Oura, Okinawa	July 13	1	0
Komi, Iriomote	August 11	6	5
Komi, Iriomote	August 12	2	7
Komi, Iriomote	August 13	0	5
Total		10	17

to be disturbed during the process (Fig. 1). Three females (case nos. 2, 5, and 6) interrupted their blood feeding because of movement of the host; 1 female (case no. 2) subsequently returned to the fish for a full feeding. The engorged females laid a batch of 10–40 eggs; most eggs hatched and developed to the adult stage.

One of us (Okudo) often exposed his hand to about 100 mature *Oc. baisasi* females in the rearing cage, but the females did not attempt to bite his hand. The females did not seem to be attracted to warm-blooded animals while confined.

Blood meal sources of *Oc. baisasi* as determined by ELISA are shown in Table 2. Ten (37.0%) blood meals of 27 tested females were determined to have fed on a fish. Two species of the oxudercine gobies, also called mudskippers—*P. argentilineatus* and *P. modestus*—are distributed in the estuaries, intertidal flats, and mangrove forests of the Ryukyu Archipelago (Murphy 1989, Clayton 1993). According to our observations, *P. argentilineatus* are very common in the mangrove forests of Oura, Okinawa, and Komi, Iriomote, and appear to be a suitable host for *Oc. baisasi*.

Seventeen (63.0%) blood meals of 27 tested females did not respond to antiserum produced by fish. The small volume of blood meal in the stomach could be the cause of the low determination rates for fish, or the female of *Oc. baisasi* might take blood not only from fish but also from other sources such as reptiles and amphibians. In this study, only 1 commercial antiserum produced from fish was used to determine blood source. Further close observation of feeding behavior and determination of blood meal sources of this mosquito species should be carried out in the field and laboratory.

In the field, it was often observed that when the crab hole was disturbed in daytime, the resting mosquitoes flew out of the hole, but did not fly far and promptly returned to the same hole or neighboring holes. Most of the resting females collected at the holes in Oura mangrove forest were gravid, having mature eggs in their abdomens. A few of

them had fresh blood meals, and others were newly emerged nulliparous. It can be assumed that *Oc. baisasi* females feed fully on a mudskipper at night. Because of the heavy weight of blood, she will land on the nearest hollow and rest for several hours until the blood meal in her stomach has been digested to a certain extent. The half-gravid female will fly to crab and mud lobster holes for oviposition and to rest until morning.

Mudskippers (Oxudercinae) are a terrestrially adaptable fish. They are exposed to air for long periods of time and rest on plant roots in tidal mudflats and mangroves at night (Clayton 1993). Because many mudskippers breed in tide pools scattered in the forest, they might be the most readily accessible source of a vertebrate blood meal for the crab hole-dwelling mosquitoes. Although the feeding habits of the 9 species of subgenus *Geoskusea* are not known, except for *baisasi* and *longiforceps*, most of these species could feed readily on a mudskipper in the tidal zone.

ACKNOWLEDGMENTS

We express sincere appreciation to Yukio Nakasone and Takashi Uchino, University of the Ryukyus, for providing information and literature on the crab and mud lobster; and to Yuko Nakazato for useful information on the mudskipper. Our appreciation also goes to Masaya Tatara and Chieko Matsumoto, Iriomote Wildlife Center, and Mikako Tamashiro for their kind collaboration. Thanks are also due to Zenyo Yamazato and Takafumi Tomoyose, Nago City Office, for giving permission to enter the important cultural asset, Oura mangrove forest. We are also grateful to Yong Hoi-Sen for his review of the manuscript.

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